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Effect of yeast probiotic supplements in the diet of young ewes on the metabolic activity of rumen microbiota

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ABSTRACT. The aim of the current study was to analyse the effect of different doses of a new probiotic (produced in Ukraine from *Saccharomyces cerevisiae*) in the feed on the production of nitrogen metabolites in the rumen, enzymatic activity of the digestive tract microbiota and growth rate of young ewes. The experiments showed that the supplementation of the probiotic EnzActive at doses of 0.4, 0.8 and 1.2% (w/w) to concentrate feed for 11–12 month old sheep had a positive effect on the production of nitrogen compounds, enzymatic activity of the rumen microbiota and animal growth intensity. It was proven that the introduction of the specified probiotic at a dose of 1.2% significantly increased the production of total nitrogen and amylolytic, proteolytic and cellulolytic activity of the rumen microbiota compared to animals that did not receive it. It was also found that the highest significant indices of animal growth rate during the experimental period were obtained for 0.8% probiotic supplementation.

Introduction

Many scientific experiments have shown that the use of fungal-based probiotics in the diet of ruminants stabilises the acidity of the rumen environment, activates microbial processes, stimulates the breakdown of fibre by ruminal microbiota, improves substrate supply to energetic and synthetic processes in tissues, as well as increases milk, meat and wool productivity of animals (Fuller, 1989; Newbold et al., 1996; Lee et al., 2003; Kutasi et al., 2004; Guedes et al., 2008; Desnoyers et al., 2009; Bąkowski and Kiczorowska, 2021; Michalak et al., 2021; Vovk et al., 2021).

Recently, probiotic supplements based on the yeast *Saccharomyces cerevisiae* have started to be widely applied in ruminant feeding practices (Ogbuewu et al., 2018; Mohammed et al., 2018; Bąkowski and Kiczorowska, 2021; Michalak et al., 2021; Vovk et al., 2021). Studies conducted in recent years demonstrated that dietary yeast probiotic supplements exerted the most pronounced positive effects on rumen digestion processes and the productivity of young ruminants, including calves, lambs and kids (Fadel and Abusamra, 2007; Lascano et al., 2009; Moharrery and Asadi, 2009; Kowalik et al., 2012; Pienaar et al., 2012; Issakowicz et al., 2013; Comert et al., 2015; Mohammed et al., 2018; Estrada-Angulo et al., 2021).

The mechanism of biological action of probiotics in ruminants has only been partially elucidated, but recent studies have indicated that it is complex and multifaceted. Latest research demonstrated that biologically active substances of probiotics stimulated the symbiotic rumen microbiota, and reticulum to the synthesis of mediators activating the functioning of digestive processes; metabolites produced by probiotics show antipathogenobacterial, immunomodulatory, anti-inflammatory and antiallergic effects in animals (Markowiak and Śliżewska, 2018;

Bąkowski and Kiczorowska, 2021; Michalak et al., 2021; Vovk et al., 2021).

The range and quality of new yeast probiotic additives to ruminant diets are increasing on the world market. Therefore, studies to determine the metabolic and production effects of improved yeast probiotic supplements in ruminant diets are of both scientific and practical importance. Based on the above, the aim of the current work was to analyse the effect of different doses of a new probiotic (produced in Ukraine from *S. cerevisiae*) in the feed on the production of nitrogen metabolites in the rumen, enzymatic activity of the microbiota in the digestive tract and growth rate of young ewes.

Material and methods

Experimental procedures

The study was conducted in 2020 at the "Grusiatychi" State Research Farm (Lviv region, Zhydachiv district, village Grusiatychi) and at the Department of Small Animals of the Institute of Agriculture of the Carpathian region of the National Academy of Agrarian Sciences of Ukraine.

Using the method of live weight and age analogues (live weight of animals at the beginning of the experiment was 38.0–38.2 kg, age: 11 months), young ewes of the Askanian meat-wool breed were assigned to 4 experimental groups, 5 animals each. The scheme of the experiment is presented in Table 1. The main diet of young ewes in the first control group consisted of meadow grass hay and standard concentrate feed that provided the animals with macro- and micronutrients and vitamins. Young ewes from the first, second and third experimental groups were administered the probiotic EnzActive in the amount of 0.4, 0.8 and 1.2% by weight of the concentrate (Table 1). AOAC International (2000) procedures were used to determine dry matter, ether extract and crude protein in the diets. The duration of the experiment was 60 days (February–March 2020). The study used the probiotic EnzActive, prepared on the basis of baking yeast *S. cerevisiae,* produced by Enzyme (Lviv, Ukraine). The content and nutritional value of EnzActive is shown in Table 4.

The composition of the basal diet fed to the experimental ewes is given in Table 2.

Table 1. Experimental design

Animal groups	Number of animals in group (heads)	Composition of daily diet
Control	5	BD
Experiment 1	5	BD + 0.4% EA by feed weight
Experiment 2	5	BD + 0.8% EA by feed weight
Experiment 3	5	BD + 1.2% EA by feed weight

BD – basal diet: 1.1 kg of meadow hay +0.5 kg of compound feed, EA – EnzActive probiotic prepared on the basis of baking yeast *Saccharomyces cerevisiae*

Table 2. Components of compound feed for experimental young ewes

Name of feed	Content, %
Corn	10
Oats	15
Barley	30
Wheat	15
Soybean meal	15
Sunflower meal	12
Monocalcium phosphate	1
Salt	1
Premix (composition is given in Table 3)	1
1 kg of compound feed contains	
dry matter, g	846
metabolic energy, mJ	10.7
crude protein, g	146
crude fat, g	32
crude fibre, g	84
calcium, g	3
phosphorus, g	6

Table 3. Premix composition

Components	Units	Quantity
Vitamins		
D_3	IU/kg	200
E	mg/kg	2000
K_3	mg/kg	20
B_1	mg/kg	230
B_2	mg/kg	880
B_6	mg/kg	300
B_{12}	mg/kg	5
C	mg/kg	3000
Calcium pantothenate	mg/kg	2000
Folic acid	mg/kg	30
Choline chloride	mg/kg	0
Niacin	mg/kg	1950
Biotin	mg/kg	3
Trace elements		
manganese	mg/kg	8000
zinc	mg/kg	6000
copper	mg/kg	1300
iodine	mg/kg	50
cobalt	mg/kg	175
selenium	mg/kg	5
filler	g	up to 1000

Table 4. EnzActive probiotic composition

	Units	Quantity
Yeast *Saccharomyces cerevisiae*	-	-
Enzyme		
xylanase	U/g	700 000
phytase	U/g	100 000
cellulase	U/g	420 000
alpha-amylase	U/g	28 000
beta-glucanase	U/g	49 000
Nutrient value of 100 g		
carbohydrates	g	43.5
sugar	g	13.8
fat	g	0.4
saturated fat	g	0.03
protein	g	43.2
raw fibre	g	0.00
sodium chloride	%	0.08
energy value of 100 g	kJ	1467.0

GMO status: yeasts did not contain genetically modified organisms and were manufactured without the use of genetic engineering methods

Feed consumption was recorded every 10 days by weighing the amount of feed and unconsumed residues on a BDU60-0404-A technical scale to the nearest 0.1 kg (Vagy AXIS Ukraine, Lviv, Ukraine) The average daily consumption per head of the tested ewes during the experiment was as follows: dry matter 1.3 kg, metabolic energy 13.2 MJ, crude protein 176 g, crude fat 39 g, crude fibre 390 g, calcium 9 g, phosphorus 5 g. At the end of the 60-day experimental period, ruminal fluid was collected from 3 ewes (from each of the 4 groups) after morning feeding using a nasopharyngeal probe; subsequently, the content of nitrogen metabolites and enzymatic activity of the microbiota was determined.

To determine the intensity of growth, ewes were weighed on an electronic 4BDU300-XB-0615 scale (Vagy AXIS Ukraine, Lviv, Ukraine), with an accuracy of 0.1 kg, at the beginning, at the end of the 1st month, and at the end of the experimental period.

Determination of nitrogen compound contents in the fluid

The concentration of total nitrogen in the rumen fluid was determined by the Kjeldahl method (Kjeldahl, 1883). The method consists in heating a sample to 360 °C with concentrated sulphuric acid to liberate the reduced N as ammonium sulphate. Non-protein nitrogen content was determined by the Kjeldahl method (Kjeldahl, 1883) in the rumen fluid centrifugate obtained after protein precipitation with 0.3 N $Ba(OH)_2$ solution and 2 ml of 5% zinc sulphate. The determination of protein nitrogen content in the rumen fluid was calculated from the difference between total and non-protein nitrogen levels.

Conway units were used to determine ammonia concentration in the rumen fluid using Nessler's reagent, after prior separation from interfering compounds by microdiffusion.

Determination of enzymatic activity of rumen fluid microbiota

Amylase activity was assayed according to Kopečný and Bartoš (1990) using soluble starch as substrate parameters in the experimental and control samples.

Determination of proteolytic activity of the rumen microflora was based on the release of amino acids detected by the ninhydrin reaction. Proteolytic activity was determined based on the difference between experimental control samples and expressed in µmol tyrosine released in 100 ml of rumen fluid in 1 min.

The cellulolytic activity of the rumen microbiota was determined by introducing filter paper strips and 15 ml of filtered rumen fluid into 30 ml test tubes. The control tubes contained the same amount of water. Control and test tubes were incubated in a TC-80 incubator (Miz-Ma, Ukraine) at 30 °C. Then, the contents of the tubes with filter paper strips were transferred to glass filters, and washed with distilled water. Glass filters with filter paper residues were transferred to an oven and dried at 105 °C. The cellulolytic activity (%) was determined by the difference between the weight of the filter paper from the experimental samples and control tubes.

Statistical analysis

Statistical processing of the obtained data was carried out using a standard MS Excel programme. Mean values and standard errors were determined for all studied traits. The statistical significance of the results was assessed using Student's test. The difference between the control and experimental groups was considered statistically significant at $P \leq 0.05$.

Results

Changes in the level of nitrogen compounds in the rumen fluid of young ewes under the influence of the probiotic are shown in Table 5.

It was found that the supplementation of the probiotic EnzActive to young ewes in the amount 1.2% by weight increased the total nitrogen production in the ruminal fluid of animals in comparison to the control group (Table 5). No effect of EnzActive probiotic supplementation was observed on the pro-

Table 5. Effect of the probiotic EnzActive (EA) on nitrogen fractions in the rumen fluid of young ewes (n = 3)

N-fractions, mg/100 ml	Animal groups			
	Control	Experiment 1	Experiment 2	Experiment 3
Total nitrogen	118.3 ± 3.4	122.5 ± 4.1	131.2 ± 4.1	134.5 ± 4.3*
Non-protein nitrogen	41.2 ± 2.1	41.7 ± 3.7	43.3 ± 4.6	42.2 ± 3.1
Protein nitrogen	72.5 ± 2.5	72.9 ± 3.9	76.5 ± 3.9	74.3 ± 2.7
Ammonia	17.6 ± 1.3	17.3 ± 1.2	15.6 ± 2.8	14.5 ± 1.5

Control – basal diet (BD): 1.1 kg of meadow hay + 0.5 kg; Experiment 1 – BD + 0.4% EA by feed weight; Experiment 2 – BD + 0.8% EA by feed weight; Experiment 3 – BD + 1.2% EA by feed weight; data are presented as mean value ± SD (standard deviation); * – significantly different from control at $P \leq 0.05$

duction of others nitrogen compounds in the ruminal fluid of animals.

Changes in the enzymatic activity of the rumen microbiota affected by the probiotic are shown in Table 6.

The data presented in Table 6 indicate the relationship between the amount of the probiotic in the concentrated feed of young sheep and an increase in the enzymatic activity of the symbiotic rumen microbiota. In addition to the stimulation of the production of nitrogen compounds by the microflora of the rumen environment, the addition of EnzActive in the tested doses to the concentrate feed of young ewes also increased the amylolytic, proteolytic and cellulolytic activity of the rumen microbiota. Compared to the control group, the highest level of microbial amylolytic activity was found in the rumen fluid of experimental groups 2 and 3, which received the bioadditive at doses of 0.8 and 1.2% (w/w) of the concentrate, respectively ($P \leq 0.001$). As for the proteolytic activity of the rumen microbiota, its increase was found in animals of all experimental groups compared to control ($P \leq 0.01$). The highest level of the cellulolytic activity of microorganisms of the rumen environment was recorded in animals from the 3rd experimental group ($P \leq 0.001$).

Table 6. Effect of the probiotic EnzActive (EA) on enzymatic activity of the rumen microbiota (n = 3)

Group	Amylolytic activity, units	Proteolytic activity tyrosine, μmol in 100 ml/min	Cellulolytic activity, %
Control	0.73 ± 0.05	2.11 ± 0.37	17.2 ± 0.43
Experiment 1	0.79 ± 0.06	2.35 ± 0.23	17.9 ± 0.52
Experiment 2	1.32 ± 0.04***	2.46 ± 0.27	18.4 ± 0.49
Experiment 3	1.55 ± 0.08***	2.79 ± 0.31**	18.9 ± 0.75***

[1] the activity was expressed as mg sugar liberated/h; Control – basal diet (BD): 1.1 kg of meadow hay + 0.5 kg; Experiment 1 – BD + 0.4% EA by feed weight; Experiment 2 – BD + 0.8% EA by feed weight; Experiment 3 – BD + 1.2% EA by feed weight; data are presented as mean value ± SD (standard deviation); **,*** – significantly different from control at $P \leq 0.01–0.001$

The data presented in Table 7 indicated that the supplementation of the probiotic EnzActive in the rations of young ewes at the studied doses exerted a positive effect on the intensity of animal growth. The highest significant gross and average daily gains in live weight over the 2-month experimental period, compared to control, were observed in animals from the 2nd experimental group ($P \leq 0.05$). Young ewes of this group received 0.8% probiotic as part of the concentrate feed.

As a result, it can be concluded that feeding young ewes with a compound feed supplemented with the active enzyme probiotic at a dose of 1.2%

Table 7. Effect of the probiotic EnzActive (EA) on body weight and growth intensity indices of ewes

Indicators	Animal groups			
	Control	Experiment 1	Experiment 2	Experiment 3
Body weight, kg				
start of the experiment	38.0 ± 1.06	38.2 ± 1.02	38.2 ± 0.65	38.0 ± 1.00
end of the experiment	42.0 ± 0.35	43.2 ± 0.55	43.6 ± 0.57*	42.6 ± 0.84
Increase				
gross, kg	4.00	5.00	5.40	4.60
average daily, g	66.7	83.3	90.0	76.7
relative, %	5.00	6.14	6.60	5.71

Control – basal diet (BD) 1.1 kg of meadow hay + 0.5 kg; Experiment 1 – BD + 0.4% EA by feed weight; Experiment 2 – BD + 0.8% EA by feed weight; Experiment 3 – BD + 1.2% EA by feed weight; data are presented as mean value ± SD (standard deviation); * – significantly different from control at $P \leq 0.05$

(w/w), compared to animals that did not receive supplementation, probably increases the production of total nitrogen by the rumen microbiota, as well as its amylolytic, proteolytic and cellulolytic activity. At the same time, a significant increase in gross and average daily live weight gains was recorded in young ewes supplemented with 0.8% (w/w) of the probiotic compared to the control group.

Discussion

In ruminants, probiotics stimulate the reproduction, vital and metabolic activity of the ruminal microbiota, inhibit the development of pathogenic microbes in the digestive tract, exert immunomodulatory effects, and increase productive qualities of animals (Ogbuewu et al., 2018; Markowiak and Śliżewska, 2018; Bąkowski and Kiczorowska, 2021; Michalak et al., 2021; Vovk et al., 2021).

Currently, the most common probiotics used as bioadditives in ruminant diets are preparations based on microscopic fungi, including baker's yeast *S. cerevisiae*.

Countries with developed sheep breeding allow application of various probiotic preparations on the basis of *S. cerevisiae* to activate ruminal digestion processes, stimulate protein biosynthesis by the rumen microbiota and increase animal production traits (Moharrery and Asadi, 2009; Pienaar et al., 2012; Issakowicz et al., 2013; Comert et al., 2015; Estrada-Angulo et al., 2021).

In recent years, in Ukraine (Lviv), Enzym has started to produce a range of probiotic, prebiotic and symbiotic supplements based on *S. cerevisiae* for the needs of animal husbandry, and the probiotic EnzActive is one of these preparations. However, the metabolic and production effects of this probiotic, when added to animal diets, have not been studied. Therefore, the aim of our research was to determine the effect of different doses of the specified probiotic in young sheep diets on the production of ammonia, total protein and non-protein nitrogen by the rumen microbiota, its amylolytic, proteolytic and cellulolytic activity, and the intensity of animal growth.

Our results regarding the activation of the production processes of various nitrogen forms and the reduction of ammonia formation in the rumen fluid after the introduction of different amounts of the probiotic EnzActive into the mixed feed are consistent with the data of other authors (Doležal et al., 2005; Guedes et al., 2008; Moharrery and Asadi, 2009; Hristov et al., 2010; Ahlam, 2011; Kowalik et al., 2012; Kamal et al., 2013; Ozsoy et al., 2013). The cited authors conducted studies in cattle, sheep and goats, and found that the use of dietary probiotic supplements based on *S. cerevisiae* stabilized rumen pH, optimized quantitative and qualitative composition of its microbiota, and stimulated its protein-synthesizing activity.

We also demonstrated that the probiotic EnzActive introduced into the feed of young ewes had a generally stimulating effect on the metabolic processes of the symbiotic rumen microbiota, especially on amylolytic, proteolytic and cellulolytic enzymatic activity. Activation of rumen fermentation processes, optimising effect on the quantitative and qualitative composition, as well as the activity of rumen microorganisms in cows, sheep and goats have been confirmed by many researchers, who applied probiotic yeast supplements in animal feed diets (Doležal et al., 2005; Guedes et al., 2008; Desnoyers et al., 2009; Hristov et al., 2010; Ahlam, 2011; Kamal et al., 2013; Ozsoy et al., 2013; Comert et al., 2015).

Regarding the production effects of the probiotic EnzActive added to the feed rations of young ewes, they are in line with the results of other researchers, who reported increased live weight gain in heifers (Lascano et al., 2009; Kowalik et al., 2012), lambs (Moharrery and Asadi, 2009; Mikulec et al., 2010; Pienaar et al., 2012; Issakowicz et al., 2013; Estrada-Angolo et al., 2021) and goat kids (Fadel and Abusamra, 2007) after dietary yeast probiotic administration.

The results of the present study have generally indicated that the probiotic EnzActive, used as an feed additive for young ewes, proved to be effective at the tested doses. EnzActive stimulated the processes of protein synthesis, enhanced amylolytic, proteolytic and cellulolytic enzymatic activity of the rumen microbiota, while inhibiting microbial ammonia production, and increasing the intensity of animal growth. The results demonstrated that based on the metabolic and production effects, the 1.2% (w/w) dose of the probiotic EA was the most optimal amount in the concentrate feed for young ewes.

Conclusions

Addition of the probiotic EnzActive at doses of 0.4, 0.8 and 1.2% to the feed of young ewes had a positive effect on the production of nitrogen compounds, enzymatic activity of the rumen microbiota and intensity of animal growth. Compared to the control group animals, 1.2% probiotic in the concentrate feed composition exerted the strongest effect on the increase of total nitrogen production, as well as the amylolytic and proteolytic activity of the rumen microbiota. The intensity of animal growth was most highly stimulated by 0.8% probiotic addition to the concentrate feed.

Conflict of interest

The Authors declare that there is no conflict of interest.

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